

Anthocyanins and Flavonols of *Chimonanthus praecox* (Calycanthaceae) as Flower Pigments

Tsukasa IWASHINA^a, Fumihiro KONTA^b and Junichi KITAJIMA^c

^aTsukuba Botanical Garden, National Science Museum,
Amakubo 4-1-1, Tsukuba, Ibaraki, 305-0005 JAPAN;

^bDepartment of Botany, National Science Museum,
Amakubo 4-1-1, Tsukuba, Ibaraki, 305-0005 JAPAN;

^cLaboratory of Pharmacognosy, Showa College of Pharmaceutical Sciences,
Higashi-tamagawagakuen 3, Machida, Tokyo, 194-8543 JAPAN

(Received on August 8, 2000)

Three anthocyanins and five flavonols in the flowers of *Chimonanthus praecox* var. *praecox* and *C. praecox* var. *grandiflorus* f. *concolor* were isolated. Anthocyanins were characterized as cyanidin 3-*O*-glucoside (chrysanthemin), acylated cyanidin 3-*O*-glucoside and cyanidin glycoside. Flavonols were identified as quercetin 3-*O*-rutinoside (rutin), quercetin 3-*O*-glucoside (isoquercitrin), kaempferol 3-*O*-rutinoside (nicotiflorin), quercetin 3-*O*-rutinoside-7-*O*-glucoside and quercetin itself, by comparisons of PC and HPLC data with authentic specimens, NMR and UV spectra, and characterization of the complete and partial acid hydrolysates. Though the major flavonoid of the species was named “meratin” and regarded as quercetin 3-*O*-diglucoside, this has been revealed to be quercetin 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside, namely “rutin” based on ¹H- and ¹³C-NMR spectra.

Key words: Anthocyanins, Calycanthaceae, *Chimonanthus praecox*, flavonols, meratin

Introduction

The genus *Chimonanthus* includes only two species, *C. praecox* (L.) Link and *C. nitens* D.Oliver, and is endemic to China. Of those species, the former is cultivated in Japan for ornament use, since the yellow flowers, inner perianth of which is dark red, bloom in winter until early spring. A flavonol glycoside isolated from the flowers of *C. praecox* as the major yellow pigment was named “meratin” (Hayashi and Ouchi 1946), and regarded as quercetin 3-*O*-diglucoside (Hayashi and Ouchi 1947). However, the red pigments of the inner perianth have not been isolated.

In this paper, the isolation and identification of the flower pigments in *C. praecox*

var. *praecox* and var. *grandiflorus* f. *concolor* are described.

Materials and Methods

Plant materials

The flowers of *Chimonanthus praecox* (L.) Link. var. *praecox* were collected in Omichi Garden, the Imperial Palace, Tokyo (TNS 697502 and 697503), and those of *C. praecox* var. *grandiflorus* (Lindl.) Makino f. *concolor* Makino in Shibata City, Niigata Pref. (TNS 697504). Voucher specimens were deposited in the National Science Museum, Tokyo (TNS).

Isolation of anthocyanins

Fresh dark red inner perianths of *C. praecox* var. *praecox* and yellow perianths of *C.*

praecox var. *grandiflorus* f. *concolor* (ca. 2.5 g, each) were extracted with 0.1% HCl in MeOH, respectively. The concentrated extracts were applied to preparative paper chromatography (PPC) with solvent systems: BAW (n-BuOH/HOAc/H₂O = 4:1:5, upper phase) and 15% HOAc. The isolated anthocyanins **A1** and **A2** were purified by Sephadex LH-20 column chromatography (solvent system: 70% MeOH + 1 drop HCl).

Isolation of flavonols

The fresh perianths of *C. praecox* var. *praecox* (2.8 g) and *C. praecox* var. *grandiflorus* f. *concolor* (1.7 g) were extracted with MeOH, respectively. After investigation of the flavonoid composition by two-dimensional paper chromatography (2D-PC) and high performance liquid chromatography (HPLC), the extracts were combined and applied to PPC with BAW, 15% HOAc and then BEW (n-BuOH/EtOH/H₂O = 4:1:2.2). Five components (**F1**–**F5**) isolated were purified by Sephadex LH-20 column chromatography (solvent system: 70% MeOH). The major component **F1** was obtained as pale yellow powder (ca. 15 mg) from EtOAc/MeOH solution.

HPLC

HPLC separation was performed with JASCO HPLC systems according to Iwashina (1996) for anthocyanins (eluent: MeCN/HOAc/H₃PO₄/H₂O = 6:8:3:83) and Iwashina et al. (1996) for flavonols (eluent: MeCN/H₂O/H₃PO₄ = 22:78:0.2).

Acid hydrolysis

Anthocyanins were hydrolyzed in 12% aq. HCl, directly heated, for 3 min., and flavonol glycosides in 12% aq. HCl, 100°C, for 30 min. Partial acid hydrolysis of the flavonols was performed in 0.1% aq. HCl:MeOH (1:1), 90°C, for 10–20 min.

Identification of anthocyanins

Anthocyanins were identified by direct PC (solvent systems: BAW, BuH (n-BuOH/HCl/H₂O = 7:2:5) and 1% HCl) and HPLC comparisons with authentic specimens, charac-

terization of acid hydrolysates, and UV-visible spectral properties.

PC, HPLC and UV-visible spectral data of isolated anthocyanins are as follows:

Cyanidin 3-*O*-glucoside (chrysanthemin, **A1**). PC: Rf 0.16 (BAW), 0.28 (BuH), 0.06 (1% HCl). HPLC: retention time (Rt) 11.10 min. UV λ max (nm): 0.1% HCl-MeOH 280, 529; +AlCl₃ 313, 360, 560. E₄₄₀/E_{max} = 25%.

Acylated cyanidin 3-*O*-glucoside (**A2**). PC: Rf 0.11 (BAW), 0.28 (BuH), 0.15 (1% HCl). HPLC: Rt 15.06 min. UV λ max (nm): 0.1% HCl-MeOH 281, 531; +AlCl₃ 314, 358, 565. E₄₄₀/E_{max} = 24%.

Identification of flavonols

Flavonols were identified by direct PC (solvent systems: BAW, BEW, 15% HOAc, 5% HOAc and Forestal (HOAc/HCl/H₂O = 30:3:10)) and HPLC comparisons with authentic specimens, UV spectra according to Mabry et al. (1970), and ¹H- and ¹³C-NMR spectra according to Iwashina and Kitajima (2000), and characterization of complete and partial acid hydrolysates. The PC, HPLC, UV spectral data, and ¹H- and ¹³C-NMR spectral data of isolated flavonols are as follows:

Quercetin 3-*O*-rutinoside (rutin = meratin, **F1**). PC: Rf 0.37 (BAW), 0.57 (BEW), 0.49 (15% HOAc), 0.34 (5% HOAc); UV - dark purple, UV/NH₃ - bright yellow. HPLC: Rt 5.06 min. UV λ max (nm): MeOH 257, 266sh, 296sh, 357; +NaOMe 273, 327, 412 (inc.); +AlCl₃ 274, 431; +AlCl₃/HCl 269, 299, 364, 400; +NaOAc 273, 326, 395; +NaOAc/H₃BO₃ 262, 297, 379. ¹H-NMR (500MHz, pyridine-*d*₅): δ 13.10 (1H, s, 5-OH), 8.37 (1H, d, *J* = 1.8Hz, H-2'), 8.13 (1H, dd, *J* = 2.1 & 10.4Hz, H-6'), 7.36 (1H, d, *J* = 8.6Hz, H-5'), 6.69 (1H, d, *J* = 1.6Hz, H-6), 6.64 (1H, d, *J* = 1.5Hz, H-8), 6.01 (1H, d, *J* = 7.0Hz, glucosyl anomeric), 5.36 (1H, s, rhamnosyl anomeric), 1.53 (3H, d, *J* = 5.2Hz, rhamnosyl Me). ¹³C-NMR (125 MHz, pyridine-*d*₅): δ (quercetin) 157.7 (C-2), 135.5 (C-3), 178.7 (C-4), 162.7 (C-5), 99.8 (C-6), 165.9 (C-7), 94.6 (C-8), 158.2 (C-9), 105.2 (C-10), 123.9 (C-1'), 117.9 (C-2'), 146.8 (C-3'), 150.7 (C-4'), 116.3 (C-5'), 122.4 (C-6'); (glucose) 104.9 (C-1), 76.1 (C-2), 78.7 (C-3), 71.3 (C-4), 77.5 (C-5), 68.5 (C-6); (rhamnose) 102.6 (C-1), 72.2 (C-2), 72.6 (C-3), 74.0 (C-4), 69.6 (C-5), 18.5 (C-6).

Quercetin 3-*O*-glucoside (isoquercitrin, **F2**). PC: Rf 0.57 (BAW), 0.73 (BEW), 0.37 (15% HOAc), 0.19 (5% HOAc); UV - dark purple, UV/NH₃ - bright yellow.

HPLC: Rt 6.57 min. UV $\lambda_{\max}$ (nm): MeOH 257, 265sh, 295sh, 359; +NaOMe 274, 330, 412 (inc.); +AlCl₃ 275, 433; +AlCl₃/HCl 269, 300, 361, 396; +NaOAc 273, 324, 393; +NaOAc/H₃BO₃ 261, 297sh, 379.

Kaempferol 3-*O*-rutinoside (nicotiflorin, **F3**). PC: Rf 0.53 (BAW), 0.70 (BEW), 0.52 (15%HOAc), 0.37 (5%HOAc); UV - dark purple, UV/NH₃ - dark greenish yellow. HPLC: Rt 7.71 min. UV $\lambda_{\max}$ (nm): MeOH 266, 297sh, 350; +NaOMe 276, 324, 399 (inc.); +AlCl₃ 274, 304, 354, 395; +AlCl₃/HCl 275, 302, 349, 392; +NaOAc 274, 313, 392; +NaOAc/H₃BO₃ 267, 296sh, 354.

Quercetin 3-*O*-rutinoside-7-*O*-glucoside (**F4**). PC: Rf 0.07 (BAW), 0.25 (BEW), 0.61 (15%HOAc), 0.53 (5%HOAc); UV - dark purple, UV/NH₃ - yellow. HPLC: Rt 2.21 min. UV $\lambda_{\max}$ (nm): MeOH 257, 267sh, 359; +NaOMe 276, 423 (inc.); +AlCl₃ 275, 437; +AlCl₃/HCl 269, 296sh, 365, 399; +NaOAc 263, 370sh, 412; +NaOAc/H₃BO₃ 261, 381.

Quercetin (**F5**). PC: Rf 0.68 (BAW), 0.77 (BEW), 0.01 (15%HOAc), 0.38 (Forestal); UV and UV/NH₃ - yellow. UV $\lambda_{\max}$ (nm): MeOH 256, 372; +NaOMe decomposition; +AlCl₃ 271, 454; +AlCl₃/HCl 265, 301sh, 360, 429; +NaOAc 274, 321, 394; +NaOAc/H₃BO₃ 260, 388.

Results and Discussion

HPLC and 2D-PC patterns of MeOH and 0.1% HCl in MeOH extracts which were obtained from the flowers of *Chimonanthus praecox* var. *praecox* and var. *grandiflous* f. *concolor* are shown in Figs. 1 and 2. Though the flavonoid compositions of the two taxa are the same, anthocyanin spots or peaks which derived from inner dark red perianth, appeared only on the chromatogram of *C. praecox* var. *praecox*, but not on that of *C. praecox* var. *grandiflorus* f. *concolor*.

The two anthocyanins **A1** and **A2** produced cyanidin and glucose, which were identified by direct PC comparisons with authentic samples, by acid hydrolysis. UV-visible spectral properties, especially bathochromic shift of Band I by the addition of AlCl₃, and $E_{440}/E_{\max}$ values showed that glucose is attached to 3-position of cyanidin. Of their cyanidin 3-*O*-glucosides, **A1** was proved to be cyanidin 3-*O*-monoglucoside (chrysanthemin, Fig. 3) by direct PC and

HPLC comparison with authentic specimen. Anthocyanin **A2** is more hydrophobic than chrysanthemin, since the peak of **A2** appears at later retention time than that of **A1** by HPLC (Fig. 1). Thus, it was presumed to be acylated cyanidin 3-*O*-monoglucoside such as cyanidin 3-*O*-galloylglucoside (Ji et al. 1992). However, further characterization of the compound could not be performed due to the shortage of the flower materials.

The minor anthocyanin **A3** appeared on the HPLC chromatogram of *C. praecox* var. *praecox*. Though the compound could not be isolated, it is also cyanidin glycoside, since acid hydrolysis of the crude extract liberated only cyanidin.

Five flavonoids except three anthocyanins appeared on the chromatograms (Figs. 1 and 2), and were isolated as powder or pure solution. The major flavonoid **F1** was obtained as pale yellow powder (yield, ca. 15 mg, ca. 3% of fresh flower weight). UV spectra of **F1** showed the presence of free 5-, 7-, 3'- and 4'-hydroxyl and a substituted 3-hydroxyl groups (Mabry et al. 1970). Quercetin, glucose and rhamnose, which were characterized by PC comparisons with authentic specimens, were liberated by acid hydrolysis. Thus, **F1** was regarded as quercetin 3-*O*-rhamnosylglucoside.

The flavonoid in the flowers of *Chimonanthus praecox* has been reported by Hayashi and Ouchi (1946, 1947). Only one flavonoid was isolated as crystals and named "meratin". The compound was regarded as quercetin 3-*O*-diglucoside by permethylation and then hydrolysis. The authentic sample of "meratin" is retained by one of authors (T. Iwashina). We now completely proved the sugar-sugar linkage of "meratin" by ¹H- and ¹³C-NMR spectra. The ¹H-NMR spectrum of "meratin" showed the presence of five aromatic (H-6, H-8, H-2', H-5' and H-6'), a glucosyl anomeric (δ 6.01, *d*, *J* = 7.0Hz), a rhamnosyl anomeric (δ 5.36, *s*) and a rhamnosyl Me (δ 1.53, *d*, *J* = 5.2Hz) protons.

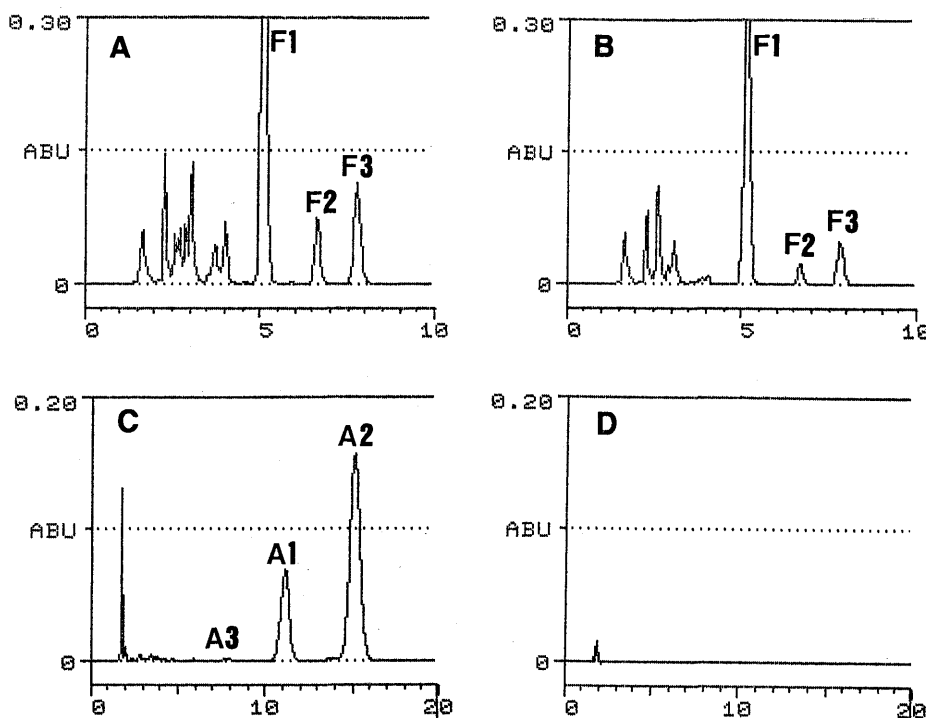


Fig. 1. HPLC pattern of MeOH and 0.1% HCl in MeOH extracts from the flowers of *Chimonanthus praecox* var. *praecox* and var. *grandiflorus* f. *concolor*. A: MeOH extracts from *C. praecox* var. *praecox*. B: MeOH extracts from *C. praecox* var. *grandiflorus* f. *concolor*. C: 0.1% HCl-MeOH extracts from *C. praecox* var. *praecox*. D: 0.1% HCl-MeOH extracts from *C. praecox* var. *grandiflorus* f. *concolor*. Eluents: A and B = MeCN/H₂O/H₃PO₄ (22:78:0.2), C and D = MeCN/HOAc/H₃PO₄/H₂O (6:8:3:83). Flow-rate: 1.0ml/min. Injection: 10 μ l. Detection: A and B = 350 nm, C and D = 520 nm. A1 = cyanidin 3-*O*-glucoside, A2 = acylated cyanidin 3-*O*-glucoside, A3 = cyanidin glycoside, F1 = quercetin 3-*O*-rutinoside, F2 = quercetin 3-*O*-glucoside and F3 = kaempferol 3-*O*-rutinoside. Other peaks are aromatic acids such as caffeoyl esters.

In the ¹³C-NMR spectrum, glucosyl C-6 (δ 68.5) appears in lower field as the case of kaempferol 3-*O*-gentiobioside (Iwashina et al. 2000) and quercetin 3-*O*-robinobioside (Iwashina, unpublished data), showing that rhamnose is attached to 6-position of glucose.

Quercetin, glucose and rhamnose were liberated by acid hydrolysis of "meratin". Since the presence of free 5-, 7-, 3'- and 4'-hydroxyl groups was indicated by UV spectral data, sugars were considered to be attached to 3-position of quercetin.

Thus, "meratin" was completely identified as quercetin 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside, namely "rutin" (Fig. 4). Moreover, "meratin" was also regarded as the same with flavonoid F1, which was isolated in this experiment, by direct PC and HPLC comparisons.

It was shown by UV spectral data that flavonoid F2 is also quercetin 3-*O*-glucoside. Quercetin and glucose were liberated by acid hydrolysis. Finally, F2 was identified as quercetin 3-*O*-glucoside (isoquercitrin, Fig. 4) by direct PC and HPLC comparisons with

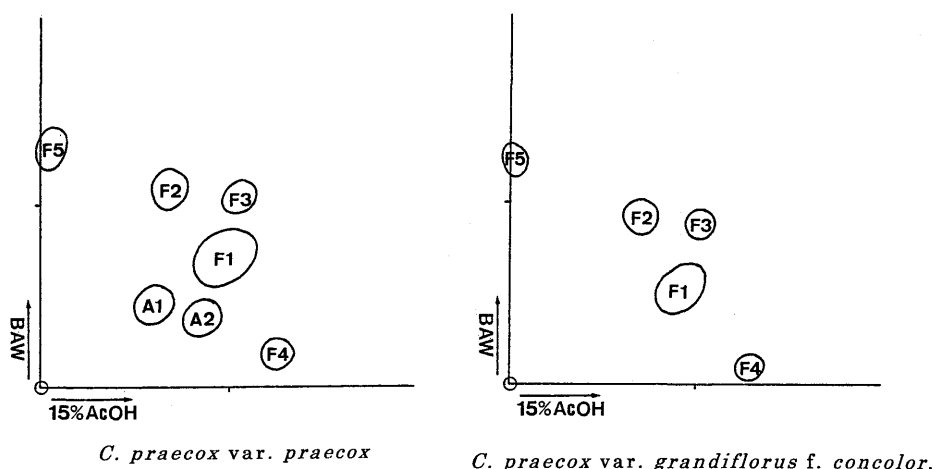


Fig. 2. 2D-PC pattern of MeOH extracts from the flowers of *Chimonanthus praecox* var. *praecox* and *C. praecox* var. *grandiflorus* f. *concolor*. **A1** = cyanidin 3-*O*-glucoside, **A2** = acylated cyanidin 3-*O*-glucoside, **F1** = quercetin 3-*O*-rutinoside, **F2** = quercetin 3-*O*-glucoside, **F3** = kaempferol 3-*O*-rutinoside, **F4** = quercetin 3-*O*-rutinoside-7-*O*-glucoside and **F5** = quercetin.

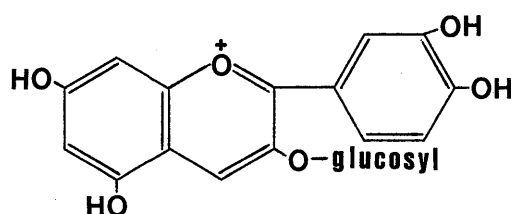
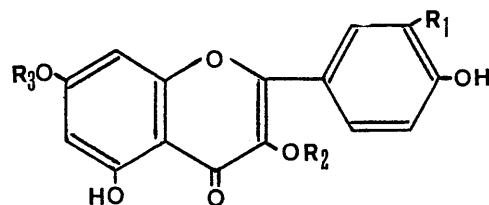


Fig. 3. Chemical structure of cyanidin 3-*O*-glucoside (chrysanthemin, **A1**).

authentic sample.

Acid hydrolysis of flavonoid **F3** produced kaempferol, glucose and rhamnose. Since UV spectra of **F3** showed the presence of free 5-, 7- and 4'-hydroxyl, and a substituted 3-hydroxyl groups, glucose and rhamnose were considered to be attached to 3-position of kaempferol. The retention times and R_fs of **F3** agreed with those of authentic nicotiflorin. Thus, flavonoid **F3** was identified as kaempferol 3-*O*-rutinoside (Fig. 4).

Minor flavonoid **F4** liberated quercetin, glucose and rhamnose by complete acid hy-



$R_1 = OH, R_2 = \text{rutinosyl}, R_3 = H$: quercetin 3-*O*-rutinoside (**F1**, rutin = meratin)
 $R_1 = OH, R_2 = \text{glucosyl}, R_3 = H$: quercetin 3-*O*-glucoside (**F2**, isoquercitrin)
 $R_1 = R_3 = H, R_2 = \text{rutinosyl}$: kaempferol 3-*O*-rutinoside (**F3**, nicotiflorin)
 $R_1 = OH, R_2 = \text{rutinosyl}, R_3 = \text{glucosyl}$: quercetin 3-*O*-rutinoside-7-*O*-glucoside (**F4**)
 $R_1 = OH, R_2 = R_3 = H$: quercetin (**F5**)

Fig. 4. Chemical structures of flavonols obtained from the flowers of *Chimonanthus praecox*.

drolysis. UV spectra of the original glycoside showed the presence of free 5-, 3'- and 4'-hydroxyl and substituted 3- and 7-hydroxyl groups. Quercetin 3-*O*-glucoside,

quercetin 3-*O*-rutinoside and quercetin 3,7-di-*O*-glucoside, which were characterized by HPLC comparisons with authentic samples, were produced as intermediates by mild acid hydrolysis.

From the results described above, flavonoid **F4** is considered to be quercetin 3-*O*-rutinoside-7-*O*-glucoside (Fig. 4).

Extremely minor flavonoid **F5** appeared as a yellow spot on the chromatogram under both UV and UV/NH₃, showing that the compound is a flavonol with free 3-hydroxyl group. UV spectra of **F5** showed the presence of free 3-, 5-, 7-, 3'- and 4'-hydroxyl groups.

Finally, flavonoid **F5** was identified as quercetin itself by direct PC comparison with authentic specimen.

As described above, the presence of three anthocyanins and five flavonols was recognized in the flowers of *Chimonanthus praecox*. Though only one flavonoid "meratin" has been isolated from the flowers of the species and characterized as quercetin 3-*O*-diglucoside (Hayashi and Ouchi 1946, 1947), our experiment revealed that it is quercetin 3-*O*-rutinoside, namely rutin. Other flavonoids of *C. praecox*, i.e., quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside-7-*O*-glucoside, kaempferol 3-*O*-

rutinoside and quercetin itself were reported for the first time.

Anthocyanins occurring in the inner dark red perianth were isolated from *C. praecox* var. *praecox*, but not from *C. praecox* var. *grandiflorus* f. *concolor* (Fig. 1D).

References

- Hayashi K. and Ouchi K. 1946. Yellow coloring matters in the flower of *Meratia praecox*. Proc. Japan Acad. **22**: 251-255.
- and — 1947. Constitution of meratin. Proc. Japan Acad. **23**: 56-57.
- Iwashina T. 1996. Detection and distribution of chrysanthemin and idaein in autumn leaves of plants by high performance liquid chromatography. Ann. Tsukuba Bot. Gard. **15**: 1-18.
- , Kamenosono K. and Yabuya T. 1996. Isolation and identification of flavonoid and related compounds as co-pigments from the flowers of *Iris ensata*. J. Jpn. Bot. **71**: 281-287.
- , López-Sáez J. A., Herrero A., Kitajima J. and Matsumoto S. 2000. Flavonoid glycosides from *Asplenium foreziense* and its five related taxa and *A. incisum*. Biochem. Syst. Ecol. **28**: 665-671.
- and Kitajima J. 2000. Chalcone and flavonol glycosides from *Asarum canadense* (Aristolochiaceae). Phytochemistry **55**: 971-974.
- Ji S.-B., Saito N., Yokoi M., Shigihara A. and Honda T. 1992. Galloylcyanidin glycosides from *Acer*. Phytochemistry **31**: 655-657.
- Mabry T. J., Markham K. R. and Thomas M. B. 1970. The Systematic Identification of Flavonoids. Springer-Verlag, Berlin.

岩科 司^a, 近田文弘^b, 北島潤一^c: ロウバイに含まれる花色素としてのアントシアニンとフラボノール

ロウバイ *Chimonanthus praecox* (L.) Link var. *praecox* の花被片は黄色であるが, 内側のものは暗赤色を呈する. 一方, ソシンロウバイ *C. praecox* var. *grandiflorus* (Lindl.) Makino f. *concolor* Makino の花被は内側までも黄色である. 本研究ではロウバイの花被に含まれるアントシアニンとその他のフラボノイド成分について分離・同定を行った. ロウバイの花から分離されたアントシアニンは2種類で, cyanidin 3-*O*-glucoside (クリサンテミン, **A1**) およびそのアシル化アントシアニン (**A2**) と定性された. また高速液体クロマトグラフィーによる分析ではもう一つの微量アントシ

アニン (**A3**) が検出されたが, これもまた cyanidin の配糖体と推定された. 一方, ロウバイおよびソシンロウバイからは共に5種類のフラボノールが分離された. 主要成分は quercetin 3-*O*-rutinoside (ルチン, **F1**) であり, その他に quercetin 3-*O*-glucoside (イソケルシトリン, **F2**), kaempferol 3-*O*-rutinoside (ニコチフロリン, **F3**), quercetin 3-*O*-rutinoside-7-*O*-glucoside (**F4**) および quercetin そのもの (**F5**) が同定された. ロウバイの花からは以前に Hayashi and Ouchi (1946, 1947) によって "メラチン" と名づけられ, quercetin 3-*O*-diglucoside と同定されたフラボノイドが唯一報告されていたが, 著者らの一人 (岩科) によって

保存されているこの“メラチン”の結晶を今回
'H-および¹³C-NMR スペクトルなどにより分析を
行ったところ、これは quercetin 3-*O*- α -L-rhamnopy-
ranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside と同定され、
“メラチン”はルチンと同一物質である事が判明
した。

(^a国立科学博物館筑波実験植物園,
^b国立科学博物館植物研究部,
^c昭和薬科大学生薬学研究室)